PB83-189670

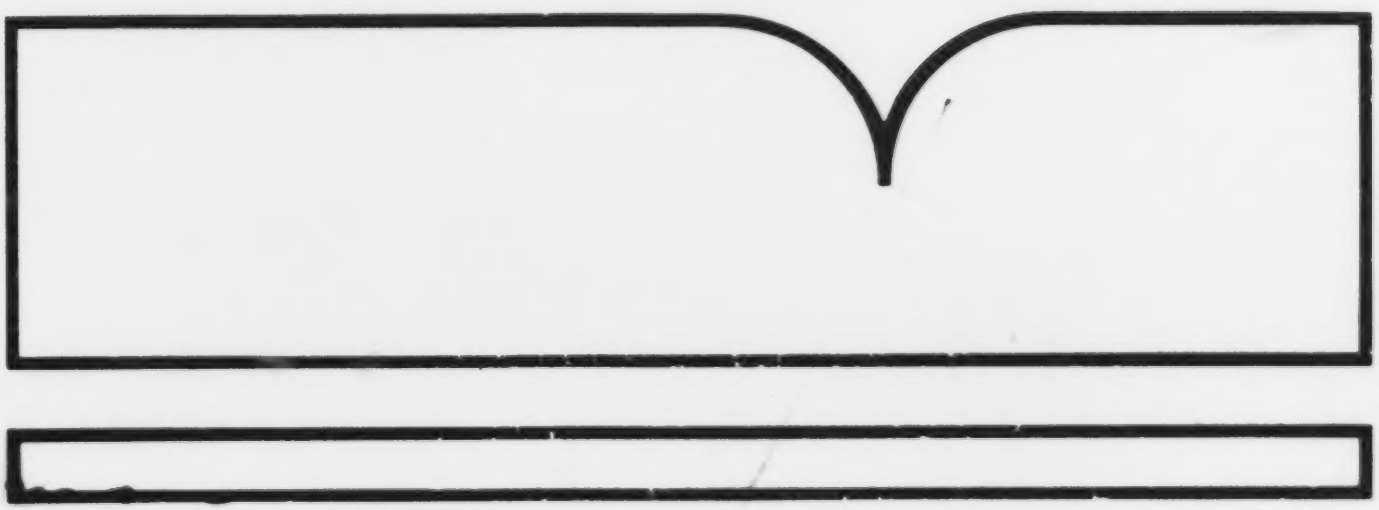
Toluene Levels in Blood and Brain of Rats during and after Respiratory Exposure

North Carolina Univ. at Chapel Hill

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

14 Jul 81



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1. REPORT NO.	2.	3
EPA-600/J-81-180	JOURNAL ARTICLE	PB83-189670

4. TITLE AND SUBTITLE	5. REPORT DATE
Journal Article: Toluene Levels in Blood and Brain of Rats during and after Respiratory Exposure	1981
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Vernon Benignus, Keith Muller, Curtis Barton, and John Bittikofer	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Neurotoxicology Division/Department of Psychology University of North Carolina Chapel Hill, NC 27514	A9RA1B
	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research and Development Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES
Published In: Toxicology And Applied Pharmacology 61, 326-334 (1981)

16. ABSTRACT
Eighty rats were exposed to 575 ppm toluene by inhalation for up to 240 min. Animals in one group were sacrificed during exposure at 15, 30, 60, 120, or 240 min. Following a 240-min exposure, groups of rats were sacrificed at 15, 30, 120, or 240 min. Blood was drawn from the vena cava of sacrificed subjects. Brains were extracted and homogenized. Blood and brain tissue were assayed for toluene by gas chromatography. One-compartment pharmacokinetic models were fitted to predict toluene levels in blood and whole brain as a function of time. Estimated asymptotes were 10.5 ppm (μg/g) for venous blood and 18.0 ppm (μg/g) for brain. Blood and brain toluene levels achieved 95% of estimated asymptotes in 53 and 58 min, respectively. Brain and blood toluene levels did not rise at significantly different rates. Though the difference was small, brain toluene level fell significantly more rapidly than toluene level in blood. In cases where other data were available in the literature on rodents or man, the results reported here agreed well with earlier data.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	10
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	

EPA-600/J-81-180
JOURNAL ARTICLE

TOXICOLOGY AND APPLIED PHARMACOLOGY 61, 326-334 (1981)

Toluene Levels in Blood and Brain of Rats during and after Respiratory Exposure

VERNON A. BENIGNUS,*·†·[1] KEITH E. MULLER,‡ CURTIS N. BARTON,† AND JOHN A. BITTIKOFER§

*U.S. Environmental Protection Agency, Neurotoxicology Division, and Departments of †Psychology and ‡Biostatistics, University of North Carolina, Chapel Hill, North Carolina 27514, and §Clinical Chemistry Laboratory, Duke University Medical Center, Durham, North Carolina 27710

Received April 11, 1981; accepted July 14, 1981

Toluene Levels in Blood and Brain of Rats during and after Respiratory Exposure. BENIGNUS, V. A., MULLER, K. E., BARTON, C. N., AND BITTIKOFER, J. A. (1981). *Toxicol. Appl. Pharmacol.* 61, 326–334. Eighty rats were exposed to 575 ppm toluene by inhalation for up to 240 min. Animals in one group were sacrificed during exposure at 15, 30, 60, 120, or 240 min. Following a 240-min exposure, groups of rats were sacrificed at 15, 30, 120, or 240 min. Blood was drawn from the vena cava of sacrificed subjects. Brains were extracted and homogenized. Blood and brain tissue were assayed for toluene by gas chromatography. One-compartment pharmacokinetic models were fitted to predict toluene levels in blood and whole brain as a function of time. Estimated asymptotes were 10.5 ppm (μg/g) for venous blood and 18.0 ppm (μg/g) for brain. Blood and brain toluene levels achieved 95% of estimated asymptotes in 53 and 58 min, respectively. Brain and blood toluene levels did not rise at significantly different rates. Though the difference was small, brain toluene level fell significantly more rapidly than toluene level in blood. In cases where other data were available in the literature on rodents or man, the results reported here agreed well with earlier data.

Toluene is a ubiquitous substance in modern environments and its potential neurotoxicity requires exploration (Benignus, 1981). Design of studies of toluene exposure is hampered by a lack of knowledge about uptake and elimination in blood and brain. This paper reports work intended to define the time course of uptake and elimination of toluene in blood and brain of rats.

Uptake and elimination of toluene from human blood have been studied widely, but the data do not agree well across investigators. It can be estimated that uptake asymptote is reached in 70 to 80 min (Veulemans and Masschelein, 1978), 15 to 20 min (Gamberale and Hultengren, 1972), and 10 to 15 min (Astrand *et al.*, 1972). Methodological and design differences make these discrepancies hard to reconcile. Elimination of toluene from human blood has been studied by Veulemans and Masschelein (1978) and by Astrand *et al.* (1972) and these reports appear to agree well despite the methodological differences.

Rates of uptake of toluene in blood and brain of mice during respiratory exposure have been reported by Peterson and Bruckner (1978). They did not fit a formal model to their data, however, and it is therefore difficult to estimate rise time constants and asymptotic values. Their means imply that asymptotic values had not yet been reached in 3 hr of exposure but no formal test of this

[1] Send requests for reprints to Vernon A. Benignus, Environmental Protection Agency/MD-58, Medical Research Building C-224H, University of North Carolina, Chapel Hill, N.C. 27514.

0041-008X/81/150326-09$02.00/0

possibility was made. Elimination of toluene in rodents is better documented than uptake (Peterson and Bruckner, 1978; Pyykko *et al.*, 1977; Sato *et al.*, 1975; Carlsson and Lindqvist, 1977).

Since data on toluene uptake in rodents during respiratory exposure are rare and not well quantified, systematic study is important. While elimination of toluene from rodent blood is somewhat better documented, formal prediction curves have rarely been fitted to data. This study was designed as a first step in a description of toluene uptake and elimination in rodents so that a comparative study in man can be systematically conducted. An important goal of this work was to devise a prediction equation which would allow formal modeling of the entire time course of toluene uptake and elimination.

METHODS

Animals. Subjects were 80 Long–Evans hooded rats supplied by Charles River (84–101 days old, mean = 95 days, SD = 6). Body weights were 305–499 g (mean = 388 g, SD = 40). All subjects were kept in an animal colony, two per cage, for a minimum of 34 days before use. An independent, but similar, group of 31 rats had been used in a pilot study to acquire preliminary data and make decisions about time of exposure, choice of control variables, and mathematical models.

Exposure. To describe the uptake of toluene, 48 rats were exposed to approximately 575 ppm toluene for 15, 30, 60, 120, or 240 min and sacrificed immediately. To study the elimination of toluene, 32 rats were exposed to a nominal 575 ppm toluene for 240 min, then exposed to laboratory air for 15, 30, 120, or 240 min, and then sacrificed. A toluene level of about 600 ppm was selected because this level represents an environmentally relevant level. A mean level of 575 ppm was actually observed. Means, standard deviations, and ranges of exposures for each time period were computed from data collected by sampling the chamber atmosphere on a 5-min interval with a Wilks Miran/80 infrared printing analyzer. The analyzer was calibrated by injections of a calculated amount of water and toluene into a closed loop. Slit width was 1 mm, path length was 21.75 m, and wavelengths were toluene, 6.17 μm, water, 6.5 μm, and reference, 3.9 μm.

Five rats were exposed simultaneously in a Rochester type chamber made of stainless steel, glass, and Teflon with a volume of 0.97 m^3 and an air flow of 0.93 m^3/min. Conditioned laboratory air was drawn through an inlet damper and an orifice at the top of the chamber. The toluene (Burdick and Jackson, distilled in glass, equivalent to HPLC grade) was introduced into the air flow between the damper and the orifice. Toluene was delivered by a syringe pump via a heated glass volatilization column.

Toluene level in the exposure chamber was stabilized before rats were inserted. Rats were placed into the chamber via a "sleeve port" in the side. The sleeve port was made of a polyethylene tube (a sleeve) of 15.2 cm diameter, attached at one end over a flanged hole in the side of the chamber. The sleeve could be unrolled and opened in order to admit one rat in an individual screen wire cage. After five rats had been inserted through the sleeve, the sleeve was rolled up and clamped. The location of the sleeve port and flanged hole was not in the stream of air to which rats were exposed. The opening of the sleeve port produced no measurable decrease in chamber concentration when sampling at the rate of once per min. The sleeve was usually open for less than 30 sec.

Sample collection. Rats were rendered unconscious by cervical dislocation. The rat was then pinned onto a dissection board and the peritoneal cavity opened. Approximately 3.5 ml of blood was drawn from the posterior vena cava, near the intersection with the renal veins, with a disposable plastic syringe. The rat was then inverted and the brain removed. Blood was dispensed into an all-glass 3-ml container which was filled so that no head space remained. Brains were placed into a weighed glass bottle with a Teflon-lined needle septum lid. Bottles were weighed again to determine the amount of tissue added. Both samples were immediately placed into an ice bath. Data were collected on the actual times required for each step in each rat, but each step was performed with maximum rapidity.

During the uptake study, rats were removed from the chamber via the sleeve port, at appropriate exposure times. During the elimination study, rats were removed in the same manner and left in laboratory air for the required time before cervical dislocation.

Sample analyses. Toluene in the blood and brain specimens was extracted into carbon disulfide and the levels were measured by gas chromatography. A 1.0-ml aliquot of blood was added to a 3-ml conical, glass extraction tube. After adding 0.5 ml deionized water to the blood specimen, the cells were lysed by vigorously shaking the tube for 2 min. A 1.0-ml aliquot of carbon disulfide, containing *p*-xylene as an internal standard, was added to the extraction tube and the mixture was agitated by an electric shaker for 5 min. This extraction mixture was centrifuged for 5 min and the lower organic layer was transferred to a 1-ml Wheaton vial. The vial

was capped and could be stored at ambient temperature for at least 4 days with no deterioration in the levels of toluene and *p*-xylene.

The components of the carbon disulfide mixture were separated in a Perkin Elmer 900 gas chromatograph wtih a flame ionization detector. A 6-ft Bentone 34 column was used. The column, injection, and detector temperatures were 50, 150, and 150°C, respectively. The nitrogen carrier gas flow rate was 20 ml/min. Toluene concentration was determined by comparing toluene to *p*-xylene peak area ratios to a calibration curve determined from prepared standards.

Brain tissue specimens were prepared by adding 1.0 ml carbon disulfide, containing *p*-xylene internal standard, to the collection bottles immediately after the tissue had been collected. At the time of sample collection, a brain sample was collected from an unexposed rat and was spiked with a toluene standard. After adding 1.0 ml of carbon disulfide, containing *p*-xylene, this sample was processed identically with the other samples. All bottles were weighed again to confirm the amount of CS_2 added. The mixtures were cooled in an ice bath and homogenized using a Brinkman Polytron homogenizer. The increase in temperature due to homogenization was nominal. The brain homogenate was then centrifuged for 5 min. The lower, carbon disulfide layer was transferred to a Wheaton vial and capped. The toluene level in carbon disulfide was measured by gas chromatography with the same parameters as used for blood. The unexposed spiked tissue sample was used as the standard for determining the toluene in the exposed tissue.

Data analysis. Pilot data from 31 rats were used for exploratory analyses to select an appropriate model to fit the data. Weight and age of the rats were not related to toluene levels and so were not used as predictors in the analyses reported here. A multicompartment pharmacokinetic model fitted the pilot data no better than a single compartment model. Consequently, a single compartment model was fitted to the data from the 80 experimental rats. Note that data from pilot rats were not included in the results of this study but served only to help make preliminary decisions.

A single-compartment mathematical model for toluene uptake (Rescigno and Segre, 1966) is:

$$C_i = \alpha(1 - e^{-\beta_1 t_{i1}})\epsilon_i \qquad (1)$$

where C_i = toluene level in tissue sample of ith rat, α = an empirical parameter representing asymptotic level, e = naperian constant, β_1 = an empirical parameter representing rate of rise (time constant), t_{i1} = exposure time (min) for the ith rat, and ϵ_i = unknown random error of measurement for the ith rat.

A single-compartment model for elimination of toluene is

$$C_i = \alpha(e^{-\beta_2 t_{i2}})\epsilon_i \qquad (2)$$

Here β_2 is the fall time constant and t_{i2} is the time from offset of exposure.

Models for uptake and elimination may be usefully combined into one model.

$$C_i = \alpha(1 - e^{-\beta_1 t_{i1}})(e^{-\beta_2 t_{i2}})\epsilon_i. \qquad (3)$$

In Eq. (3) only three empirical constants must be found for uptake and elimination data, since the α is the same for both models. For rats sacrificed during the uptake phase, t_{i2} is the time required to produce cervical dislocation and approaches zero. Under these conditions, Eq. (3) resolves to the simple uptake model, Eq. (1). For rats sacrificed during the elimination phase, t_{i1} is a large constant equal to the exposure time. In this case the term $(1-e^{-\beta_1 t_{i1}})$ approaches unity and Eq. (3) resolves to Eq. (2), the simple elimination model. In the general case neither t_{i1} nor t_{i2} is zero. The term $(1-e^{-\beta_1 t_{i1}})$ is the proportion of asymptote attained at time t_{i1} and the term $e^{-\beta_2 t_{i2}}$ is the proportion of the attained concentration which remains at elimination time t_{i2}. The model allows the prediction of toluene levels for any combination of exposure and elimination times.

This type of data has frequently been expressed as logarithms of concentration. The range and distribution of values make this convenient. Pilot data indicated a positive correlation between means and variances of raw concentrations. The logarithmic transformation also helped provide homogeneity of variance and normally distributed errors which are needed for model fitting. After logarithmic transformation, Eq. (3) becomes:

$$\ln C_i = \ln \alpha + \ln (1 - e^{-\beta_1 t_{i1}}) - \beta_2 t_{i2} + \ln \epsilon_i. \qquad (4)$$

where each $\ln \epsilon_i$ is assumed to be an independent and identically distributed normal random variable with zero mean and unknown (but common) variance. Since (4) is inherently nonlinear, iterative least-squares procedures are required to estimate the parameters (Gallant, 1975b). A procedure in the Statistical Analysis System (Helwig and Council, 1979) was used for this purpose. In order to compare model parameters for blood and brain data (a multivariate question), the technique of seemingly unrelated nonlinear regression (Gallant 1975a) was employed. Significance tests used large sample approximations (Gallant, 1975b; Burguete and Gallant, 1980).

RESULTS

Descriptive

During seven exposure sessions in which chamber concentration was logged, the mean toluene level, during the first 15 min, was 586 ppm (SD = 51, range = 532 to 686).

The average toluene level over 240 min was 566 ppm (SD = 11, range = 547 to 582). These were samples from actual exposures of individual rats. No effort was made to account for exposure level differences in the model fitting because in pilot data too little of the response variance was accounted for by this variable. Actual exposure times were all accurate to less than ±1%.

During the uptake phase, the mean time required to produce cervical dislocation, from the time of exposure offset, was 17 sec (SD = 6, range = 10 to 39). Mean time required to collect blood samples was 84 sec (SD = 36, range = 42 to 150). To collect brain samples required a mean time of 138 sec (SD = 36, range = 78 to 210). The number of subjects at each exposure time was 9 except for 30 min ($n = 8$) and 240 min ($n = 13$). Eight subjects per group were studied for all elimination times.

Model Fitting

Fitting of model parameters in Eq. (4) was performed for a three-parameter model and for a two-parameter model for both blood and brain. The two parameter model was fitted with β_1 and β_2 set equal to test the hypothesis of equality of rise and fall time constants. The results of this procedure are shown in Table 1. In Table 1, $r^2_{y\hat{y}}$ is the squared correlation between the predicted and observed log toluene levels. The SS residual column gives the residual sum of squares for each model. The smaller this number is, the better the model fits. In both blood and brain models, significantly better fit accures when $\beta_1 \neq \beta_2$, i.e., the rise and fall time constants are allowed to differ. Note the high correlations between predicted values from the model and the residuals after prediciton ($r_{y,res}$) for the two-parameter models, indicative of systematic errors of prediction. Also note the corresponding near zero correlations for the three-parameter models. These indicate that the three-parameter models are strongly preferred.

The results of significance tests comparing the blood brain models are given in Table 2. For each line in Table 2, one model was fitted. The most complex model assumes that rise rates, fall rates, and asymptotes are all distinct for blood and brain. The most complex model features and its associated residual sum-of-squares are listed in the bottom-most line of Table 2. The simplest model assumes that rise rates, fall rates, and asymptotes are all the same for blood and brain. The simplest model parameters and its associated residual sum-of-squares are listed in the top-most line of Table 2. To test whether a significant reduction in error sum-of-squares was achieved by fitting the six parameter model rather than the three parameter model, an approximate χ^2 test was computed. In the first line 273.9 − 160.0 = 113.9 is approximately χ^2 with 6 − 3 = 3 degrees of freedom. Since this overall test was significant ($p < 0.0001$) each parameter pair was tested individually. Asymptotic values for brain were significantly higher than for blood. Rise rates did not differ significantly but fall times were marginally different.

As a result of the significance tests, the final pair of equations chosen for predicting toluene levels was:

$$\widehat{\ln C_i} = \ln 10.46 + \ln (1 - e^{-0.0556t_{i1}}) - 0.0104t_{i2} \quad (5)$$

for venous blood and

$$\widehat{\ln C_i} = \ln 17.99 + \ln (1 - e^{-0.0514t_{i1}}) - 0.0116t_{i2} \quad (6)$$

for brain. The particular values for α, β_1 and β_2 were taken from Table 1. The values for β_1 for blood and brain were 0.0556 and 0.0514, respectively. While these did not differ significantly, the exact values were retained for prediction purposes.

Plots of the lines of best fit for blood and brain as estimated from Eq. (5) and (6) with

TABLE 1

MODEL PARAMETER ESTIMATES AND TESTS FOR BLOOD AND BRAIN

Number of parameters	α	β_1	β_2	$r^2_{y\hat{y}}$	r_{y,y_m}	SS residual	F
Blood							
3	10.46	0.0556	0.0104	0.80	−0.01	11.14	74.6 $p < 0.0001$
2	16.48	0.0135	—	0.71	−0.51	21.93	df = 1,77
Brain							
3	17.99	0.0514	0.0116	0.83	−0.01	11.77	55.9 $p < 0.0001$
2	26.95	0.0144	—	0.76	−0.44	20.31	df = 1,77

the observed means and standard deviations of groups superimposed are shown in Figs. 1 and 2.

According to the particular estimates of Eqs. (5) and (6) (see Fig. 3) the asymptotic value of toluene for blood is 10.5 ppm (μg/g) and for brain is 18.0 ppm (μg/g) (see Table 1 for α). These equations can predict the time at which toluene levels reach any arbitrary percentage of asymptote. To reach the 95% level during uptake required 53 min for blood and 58 min for brain. During elimination, the 5% level was reached in 288 min in blood and in 258 min in brain after cessation of exposure. Brain toluene level fell significantly faster than blood toluene level.

DISCUSSION

The rise times of toluene in blood and brain compare favorably with those reported by Peterson and Bruckner (1978), but differences in detail are present. Their data (in mice) left some uncertainty as to whether asymptotic value had been reached in 180 min (longest exposure). The data reported here were fitted to a pharmacokinetic model in which the asymptotic value was a parameter. Examination of models for blood and brain in the present data indicated that 95% asymptote during acquisition would be reached in only 53 and 58 min for blood and brain, respectively. The difference could be

TABLE 2

SIGNIFICANCE TESTS FOR EQUIVALENCE OF BLOOD AND BRAIN MODELS FOR TOLUENE ACQUISITION AND ELIMINATION

Associated hypothesis	Parameters in models	SS residual	χ^2	df	p
Overall two models are the same	α, β_1, β_2	273.9	113.9	3	<0.0001
Asymptotes are the sames	$\alpha, \beta_{11}, \beta_{12}, \beta_{21}, \beta_{22}$	230.4	70.4	1	<0.0001
Rise times are the same	$\alpha_1, \alpha_2, \beta_1, \beta_{21}, \beta_{22}$	160.2	0.15	1	<0.700
Fall times are the same	$\alpha_1, \alpha_2, \beta_{11}, \beta_{12}, \beta_2$	163.9	3.92	1	<0.048
No parameters were the same	$\alpha_1, \alpha_2, \beta_{11}, \beta_{12}, \beta_{21}, \beta_{22}$	160.0	—	—	—

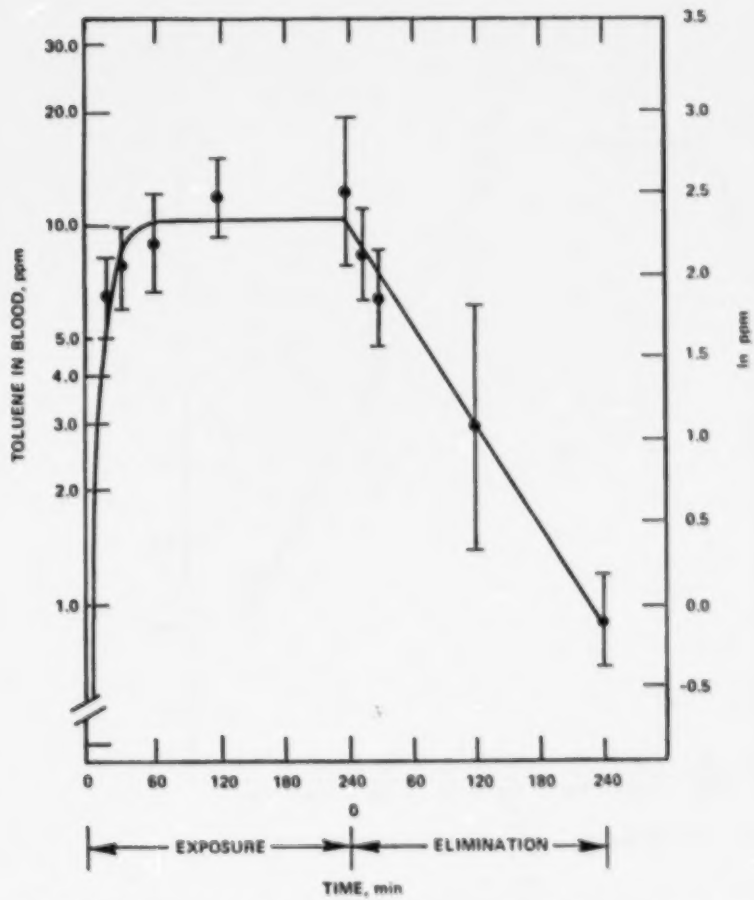

FIG. 1. Toluene uptake (inhalation) and elimination in venous blood for 575 ppm toluene exposure levels. Solid line shows estimated line of best fit for logarithmic transformed single-compartment models of Eq. (4). Values of parameters are given in Table 1. Points are group means ± 1 SD.

due to species, of course. In the present data the means of groups for longer exposure times were higher than predicted asymptote in both blood and brain (Figs. 1 and 2). This was probably random variation, but could be construed as an indication that a multicompartment model ought to have been fitted. Predicted values correlated highly with the actual data ($r = 0.89$ for blood data and $r = 0.91$ for brain data) (Table 1). With correlations as high as this, it is not probable that a multicompartment model is required. Thus, it appears that the asymptotes and times to reach them are accurately estimated by Eqs. (5) and (6).

As best as can be determined from their graphs, the elimination rates of toluene from blood and brain in this study agree very well with Peterson and Bruckner's (1978) for mice, declining to 50% of maximum in 60 min in both studies. In both studies brain showed a slightly faster elimination rate than did blood, a result which was statistically significant in the data reported here.

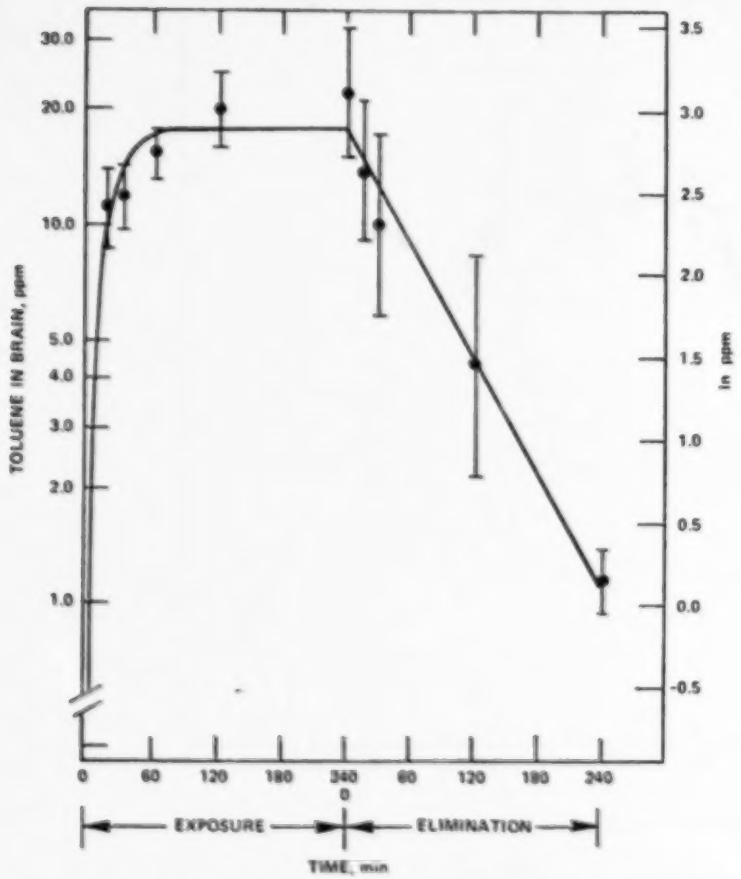

FIG. 2. Toluene uptake (inhalation) and elimination in whole brain for 575 ppm toluene exposure levels. Solid line shows line of best fit for logarithmic transformed single-compartment models of Eq. (4). Values of parameters are given in Table 1. Points are group means ± 1 SD.

If elimination rates are the same in mice and rats, it is unlikely that uptake rates would differ radically between the two species.

Levels of toluene in brain of rats, reported by Carlsson and Lindqvist (1977), for 60 min exposure to 500 ppm toluene were 15 ppm. This compares very well with the estimated 17 ppm reported here for 60 min, 575 ppm exposures. Level of toluene in blood of rats, reported by Sato *et al.* (1975), for 60 min after cessation of a 120 min exposure to 550 ppm toluene was about 3.2 ppm. In the data reported here, a corresponding value for slightly higher exposure level was 10 ppm. Note that the levels from Sato *et al.* (1975) had to be estimated graphically and the analytic methods were different.

Data on toluene uptake rates in blood of humans imply that asymptotic values may be reached in blood in as short a time as 15 to 20 min (Gamberale and Hultengren, 1972; Astrand *et al.*, 1972). Veulemans and Masschelein (1978), however, presented data which indicated much longer times. Comparisons of rat data to that of humans must await a study explicitly designed to measure uptake rates in man.

Gamberale and Hultengren (1972) ex-

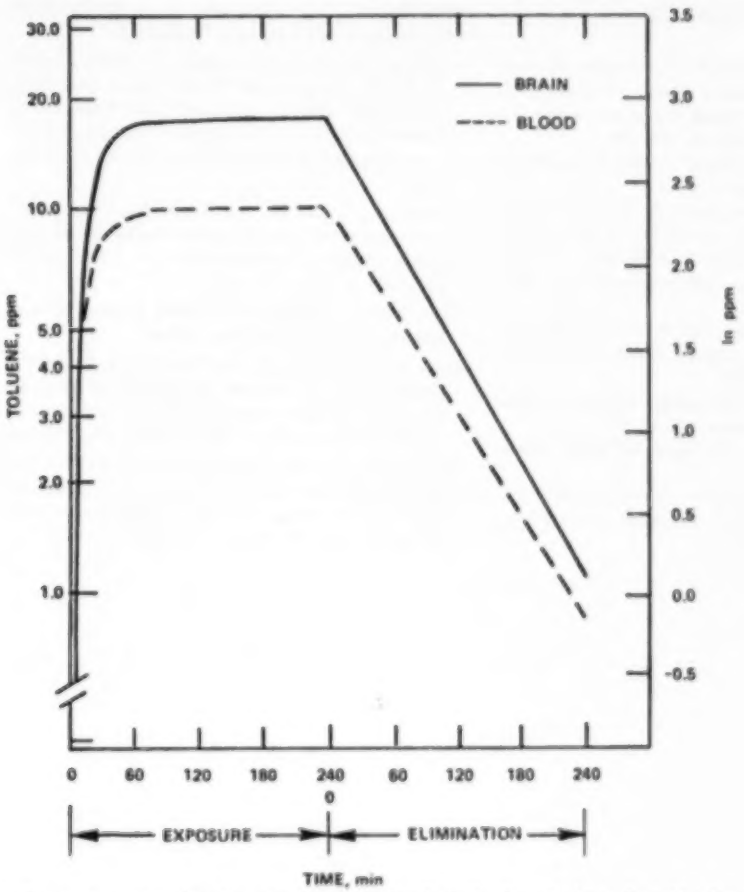

FIG. 3. Comparison of predicted uptake (inhalation) and elimination of toluene from blood and brain for 575 ppm toluene exposure levels.

posed humans to 500 ppm toluene. They reported data which can be used to crudely estimate the toluene asymptotic level in venous blood in their subjects as 12 ppm which compares with an asymptotic level of 10.5 ppm in rats which was found in this study. Considering that the ppm level in Gamberale and Hultengren's data was estimated from their report of alveolar air levels by graphic methods, the agreement is very close.

Uptake and elimination of toluene in blood and brain tissue of rats were determined as functions of time according to a one-compartment pharmacokinetic model. The model fitted accounted for most of the variance. In cases where comparable data are available in the literature, the results agreed well with this study. Thus it appears that the estimates of rise and fall time constants and asymptotic levels of toluene reported here are accurate for the rat.

ACKNOWLEDGMENTS

The authors wish to thank Thomas Williams and Marcus Vann for their help with the exposure facilities, Barbara Benton for her help with the gas chromatography, and Judith Graham and Dave Davies for their help in the design of the exposure regimen.

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